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Indigenous synthetic bacterial community alleviates continuous cropping obstacles in tomato by reshaping rhizosphere microbiome and multitrophic microbial interactions

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Abstract

Continuous cropping obstacles (CCOs) severely constrain the sustainable production of tomato, primarily by disrupting rhizosphere microecology and ultimately reducing productivity. Synthetic microbial communities (SynCom) are considered an effective strategy to alleviate CCOs; however, their ecological mechanisms remain unclear. In this study, we isolated indigenous plant growth-promoting bacteria from the rhizosphere of long-term continuously cropped tomato in Northwest China and assembled them into a functionally complementary SynCom. The effects of this SynCom on tomato growth were systematically evaluated in greenhouse experiments. The results showed that SynCom significantly increased soil available phosphorus content and urease activity, thereby promoting tomato yield and quality formation. Meanwhile, it reshaped rhizosphere microbial community structure by enriching beneficial taxa, including *Bacillus*, *Trichoderma* and *Colpoda*, and enhanced cross-kingdom microbial interactions, particularly strengthening the bacteria–protist network. Mantel-test and random forest analysis revealed that the protistan community exhibited the highest correlation and contribution to tomato yield and quality. Structural equation modeling (SEM) further demonstrated that SynCom exerted both direct and indirect positive effects on yield, whereas its promotion of quality was entirely indirect, mediated through improved plant growth, soil properties and microbial feedback. Overall, these findings reveal that indigenous SynCom alleviates CCOs by restructuring rhizosphere multitrophic microbial networks, offering novel insights for the development of indigenous-based microbial inoculants and the promotion of sustainable horticulture production in protected facilities.

Keywords: Continuous cropping obstacles (CCOs) · Synthetic microbial communities (SynCom) · Structural equation modeling (SEM) · Rhizosphere microbiome · Multitrophic microbial interactions

1. Introduction

43 Soil, the foundation of crop production, is a vital component of terrestrial
44 ecosystems. Anthropogenic activities frequently induce soil degradation, manifesting
45 as salinization, acidification, and microecological dysbiosis (Hassan et al. 2016).
46 Continuous cropping obstacles (CCOs)—also known as replant disease or soil
47 sickness—refer to the decline in crop yield and quality, increased pest and disease
48 incidence, and deterioration of soil quality and biodiversity that occur when the same
49 or closely related crop species are repeatedly cultivated on the same land, despite
50 conventional agronomic management (Li et al. 2018; Chen et al. 2020; Wang et al. 2020;
51 Xiang et al. 2022). Long-term continuous cropping has been shown to significantly
52 reduce soil available nutrients (Li et al. 2019), disrupt the stoichiometric balance of C,
53 N, and P (Yu et al. 2017), and suppress key soil enzyme activities (urease, phosphatase,
54 and sucrase). It also exacerbates secondary salinization and physicochemical
55 deterioration, ultimately culminating in CCOs (Song et al. 2022; Zeeshan et al. 2023).
56 Furthermore, CCOs further influence soil microbial community structure and diversity
57 (Nayyar et al. 2010). For example, they reduce the abundance of beneficial taxa (e.g.
58 *Bacillus*) and increase harmful taxa (e.g. *Fusarium*). (Zeng et al. 2020; Li et al. 2022a).
59 Similar reductions in bacterial diversity, beneficial bacterial abundance, and overall
60 microbial richness have also been reported in continuously cultivated tobacco, wheat,
61 and soybean soils (She et al. 2017; Liu et al. 2020; Liu et al. 2021).

62 Tomato (*Solanum lycopersicum* L.), an annual herbaceous crop belonging to the
63 Solanaceae family, is one of the most economically important and widely cultivated
64 vegetable crops worldwide because of its high nutritional value, particularly its
65 lycopene content, and substantial commercial importance (Gibon et al. 2025). China
66 leads the world in both tomato acreage and production, with the crop serving as a
67 cornerstone of the nation's facility horticulture sector (Wang et al. 2017; FAOSTAT,
68 2025), particularly in the distinctive agro-ecological zones of Northwest China (e.g.,
69 Xinjiang and Inner Mongolia) (Zhang et al. 2026). In response to escalating market
70 demand and intensification of production, long-term continuous monoculture has
71 become a predominant practice to maximize land-use efficiency and supply stability
72 (Yan et al. 2024; Hollingsworth et al. 2020). However, this practice invariably triggers
73 CCOs, which have emerged as a critical bottleneck jeopardizing the green, safe, and
74 sustainable development of China's protected horticulture industry (Wang et al. 2024).

75 In recent years, plant growth-promoting microorganisms (PGPM) have emerged
76 as a key biological strategy to mitigate CCOs, owing to their capacity to improve
77 rhizosphere microbiota, facilitate nutrient acquisition, and suppress soilborne
78 pathogens (Teiba et al. 2024; Chen et al. 2022). However, single-function strains are
79 often constrained by complex soil environments, niche competition, and environmental
80 stresses, which compromise their colonization ability and functional stability in the
81 field, thereby limiting their growth-promoting and disease-suppressing effects (Kong
82 and Liu 2022). To overcome this limitation, synthetic microbial communities
83 (SynCom)—artificially assembled from multiple functional strains—have received
84 widespread attention in recent years. SynCom integrates the functions of different
85 microorganisms in nutrient conversion, pathogen antagonism, and induction of
86 systemic resistance, achieving inter species synergy and niche complementarity,

thereby significantly improving the stability and ecological adaptability of microbial inoculants. Compared with single strains, SynCom not only amplifies plant growth promotion and disease suppression but also improves rhizosphere colonization efficiency and sustained functionality through metabolic cross-feeding and resource partitioning, while exhibiting greater resilience to environmental perturbations (Shayanthan et al. 2022; Martins et al. 2023). More importantly, the role of SynCom is not limited to its own functions, but can also reshape the indigenous microbial community structure in plant rhizosphere, thereby establishing a healthier and more resilient rhizosphere ecosystem. For example, a SynCom constructed from desert plant rhizosphere isolates remained functional in the presence of natural soil microbial communities, significantly improving tomato salt tolerance and showing detectable interactions with indigenous microbes (Schmitz et al. 2022). Furthermore, recent evidence demonstrates that SynCom constructed from multiple plant promoting rhizosphere bacteria can significantly promote tomato growth and induce systematic changes in the rhizosphere bacterial community, especially affecting the composition and abundance of rare bacterial communities, while maintaining relatively stable colonization ability (Nicotra et al. 2026).

Although previous studies have confirmed the significant potential of PGPM and their derived SynComs in promoting plant growth, suppressing soilborne diseases, and regulating rhizosphere microecology, current research on SynCom-mediated CCO mitigation remains largely confined to model plants or non-indigenous strain combinations, and the underlying mechanisms remain unclear. Especially in the typical facility agriculture areas of northwest China, there is still limited research on the systematic excavation and construction of SynCom for indigenous advantageous microbial resources, and the analysis of their comprehensive impact on tomatoes. Based on this, this study screened indigenous bacteria with both growth promoting and biocontrol functions from the roots of tomatoes that have been continuously cultivated in northwest China for a long time, constructed a functionally complementary bacterium SynCom, and evaluated its effects on tomato growth, fruit yield, and quality through greenhouse experiments. Concurrently, high-throughput sequencing technology is employed to decipher how SynCom inoculation reshapes the tomato rhizosphere soil microbial community, thereby further elucidating the potential mechanisms underlying CCOs alleviation. The findings of this research are expected to provide a crucial theoretical foundation for developing efficient and stable microbial inoculants derived from indigenous microbial resources and for advancing their application in sustainable facility crop production.

2. Materials and Methods

2.1 SynCom Used in This Study

The synthetic community used in this study comprised six indigenous bacterial strains: *Enterobacter hormaechei* FN0603, *Pseudomonas brassicacearum* BP16, *Massilia niabensis* M20, *Priestia megaterium* fHhLee0B1, *Chryseobacterium* sp.

aHhLer0J1, and *Azospirillum brasilense* JN25. Their 16S rRNA gene sequences have been deposited in the NCBI database under the accession numbers OP326194, MG887164, PZ193920, PZ193921, PZ193922, and PZ193923, respectively. For a comprehensive description of the SynCom construction methodology, please refer to our previous publication (Guo et al. 2026).

2.2 Experimental Design and Sample Collection

The tomato cultivar used in this experiment was the Hanmu series “Kouershuang” (Chifeng Herun Agricultural High-tech Industry Development Co., Ltd., Inner Mongolia, China), and the seedling substrate was a commercial Danish Pindstrup substrate (Pindstrup Mosebrug A/S, Ryomgaard, Denmark). To evaluate the effects of SynCom on tomato growth, yield, and quality under a four-cycle continuous-cropping system, we conducted a greenhouse experiment from October 2022 to August 2024. Continuous cropping obstacles were observed to emerge in May 2024. In this experiment, two treatments were also set up, one SynCom inoculated and another non-inoculated to nurse seedlings. A randomized complete block design was adopted, with five replicates per treatment. To prevent cross-contamination between treatments, transparent plastic barriers were buried to a depth of 20 cm between adjacent plots. Seedlings of each treatment were transplanted in five blocks (10 m × 6 m for each) random distributed in a greenhouse located at City Hohhot of China (111°45'5"E, 40°39'48"N), respectively. Two rows planting was adopted to plant with a within-row spacing of 0.5 m and between-row spacing of 1.2 m. All lateral shoots were removed to maintain single-stem pruning. Water and fertilizer were delivered by a drip irrigation system. When grew to five flower trusses, tomato plants were topped. The greenhouse soil was Heilu soil (a calcareous dark loess soil), with an EC of 525 µS/cm and a pH of 8.16. The tomato cultivar of 1320 was used in the experiment.

Whole tomato plants were destructively sampled at the seedling (S), flowering-fruiting (F), and ripening (R) stages using a five-point sampling method. To minimize edge effects, border rows were avoided, and plants with uniform growth were selected for sampling. Bulk soil loosely attached to the roots was gently shaken off, and the roots with adhering rhizosphere soil were placed into sterile sampling bags and transported to the laboratory under low-temperature conditions for further processing. Rhizosphere soils from each treatment were thoroughly mixed and divided into three subsamples. Two subsamples were passed through 2 mm sieve (10 mesh) for the analysis of physicochemical properties and microbial community structure, and were stored at -20°C and -80°C, respectively. The third subsample was passed through 0.425 mm sieve (40 mesh) for the determination of soil enzyme activities and stored at -80°C until analysis. The growth parameters of tomato plants are presented in Figure S1, while fruit yield and quality were measured at the full fruiting stage, when the third truss was fully ripe during the maturity stage; the results are shown in Table S1.

2.3 Determination of Physico-chemical Soil Properties and Enzyme Activity

All the rhizosphere soil samples were analyzed for pH, electronic conductivity (EC), organic matter (OM), available nitrogen (AN) and available phosphorus (AP). The pH (HJ 962-2018) and EC (HJ 802-2016) values were measured with a pH-conductivity meter (PHS-3C, Inesa Scientific Instrument Co., Ltd., Shanghai, China) and an electrical conductivity meter (DDSJ-308A, Inesa Scientific Instrument Co., Ltd., Shanghai, China), respectively. The contents of OM, AN and AP were measured using the potassium dichromate sulfuric acid heating method, alkali diffusion method and molybdenum blue method, respectively (Bao SD 2000). The activities of five soil enzymes were determined using the particular kits (Suzhou Grace Biotechnology Co., Ltd., Suzhou, China), No. G0301W for urease, No. G0307W for dehydrogenase, No. G0303W for catalase, No. G0302W for sucrase and No. G0304W for acid phosphatase, respectively.

2.4 Assessments of Microbial Community Composition and Diversity

The total DNA of all soil samples was extracted using a FastDNA® Spin Kit for Soil (MP Biomedicals, Solon, OH, USA) according to the manufacturer's protocols. Universal primer pairs were used to amplify the V3-V4 region of 16S ribosomal RNA genes in bacteria (Liu et al. 2016) (338F: 5'-ACTCCTACGGGAGGCAGCA-3', 806R: 5'-GGACTACHVGGGTWTCTAAT-3'), the ITS1(b) region of fungi (Orgiazzi et al. 2012) (ITS1F: 5'-CTTGGTCATTTAGAGGAAGTAA-3', ITS2R: 5'-GCTGCGTTCTTCATCGATGC), and the V4 region of 18S rRNA gene in protists (Bass et al. 2016) (TAReuk454FWD1F: 5'-CCAGCASCYGCGGTAATTC-3', TAReukREV3R: 5'-ACTTTCGTGATTYRA-3'). Amplicons were sequenced with the Illumina NovaSeq 6000 Platform (Illumina Inc.) using the paired-ends model (PE300) at Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). The raw sequencing data has been deposited in the NCBI Sequence Read Archive, with accession numbers PRJNA1442407, PRJNA1442661 and PRJNA1442683, respectively.

To cross-reference whether the 16S rRNA gene sequences can be found in microbiome sequences of the rhizosphere of the SynCom-treated plants, the approach described by Xu et al. (2023) and Qiao et al. (2024) was followed with slight modifications. Briefly, the full-length 16S rRNA gene sequences of the strains were determined and aligned against the ASV sequences derived from the raw sequencing data using a local BLASTN program (Fagnano et al. 2025) (<http://www.ncbi.nlm.nih.gov>, version 2.16.0+). Sequences showing 100% identity were designated as target ASVs (Xu et al. 2023), and their relative abundances were used to estimate the abundance of the corresponding strains in the rhizosphere microbiome. This strict identity threshold was applied to avoid overestimating the percentage of matching sequences, due to shorter length of 16S rRNA amplicon sequences as compared to whole 16S rRNA sequence of the inoculated strains.

2.5 Processing of Sequencing Data

The Quantitative Insights into Microbial Ecology software (QIIME 2; v2022.2) was used to optimize raw sequences and perform quality control for bacteria, fungi, and protists (Bolyen et al. 2019). QIIME 2 plugin DADA2 was used to remove chimeric sequences, correct background noise and merge paired-end reads to tags, and obtain a table of frequencies and representative sequences. Subsequently, the singletons have been removed and then clustered into amplicon sequence variants (ASVs) at 97%

threshold using the vsearch pipeline (Callahan et al. 2016). Sequences annotated as "chloroplast" and "mitochondria" were removed from all samples. Following previous studies (Dupont et al. 2016; Oliverio et al. 2020), sequences belonging to Rhodophyta, Streptophyta, Metazoa, Fungi, and unclassified Opisthokonta sequences were deleted to generate a comprehensive ASV taxonomic table for protists for subsequent analyses. To minimize the impact of sequencing depth on subsequent Alpha and Beta diversity analyses, all sample sequences were rarefied. Taxonomic assignment of ASVs for bacteria, fungi, and protists was performed by aligning against the SILVA ribosomal RNA database project (v138) (<https://www.arb-silva.de/>) (Quast et al. 2013; Yilmaz et al. 2014), the UNITE 9.0 database (v9.0) (<https://unite.ut.ee>) (Nilsson et al. 2019) and the Protist Ribosomal Reference database (PR2, v5.0.1) (https://github.com/vaulot/pr2_database) (Guillou et al. 2013), respectively, with a confidence threshold of 70%.

2.6 Bioinformatic Analysis

Alpha diversity indices (Shannon, Chao 1 and Pielou_e, etc.) were calculated using the mothur software (Schloss et al. 2009) (<http://www.mothur.org/wiki/Calculators>), and differences between groups were assessed using Student's t-test. Beta diversity was evaluated through the principal-coordinate analysis (PCoA) based on the Bray–Curtis distance was performed to evaluate the similarity of microbial community structures among different treatments, combined with the ANOSIM non-parametric test to determine whether the differences were significant. Furthermore, the Wilcoxon rank-sum test for intergroup differences was employed to compare species among microbial communities under different treatments, assess the significance level of species abundance differences, and obtain information on species with significant differences. The bioinformatics analyses of the above data were all performed on the Majorbio Cloud Platform (<https://cloud.majorbio.com>) (Xiao et al. 2024).

The “ggClusterNet” package (version 0.1.0) (Wen et al. 2022), in conjunction with previous studies (Harrell and Dupont 2019), was used to construct a robust microbial co-occurrence network based on Spearman's correlation analysis ($r > 0.7$, $P < 0.01$) in the R environment (v4.1.3), using ASVs with relative abundances greater than 0.1%, to evaluate interactions among microbial communities. Subsequently, inter-domain ecological networks (IDEN), including bacteria–fungi, bacteria–protists, and fungi–protists interactions, were further constructed (Liu et al. 2025). The “linkET” package (Huang et al. 2021) was employed to perform Mantel-tests to explore the relationships between rhizosphere microbial communities at different growth stages of tomato and their effects on yield, quality, key fruit metabolic enzyme activities, and soil nutrients. The “RandomForest” package (Li et al. 2023) was used to predict the contributions of rhizosphere microbial communities to tomato yield and quality. By summing the percentage increase in mean squared error (%IncMSE) across different microbial groups, their overall contribution to predicting yield and quality can be evaluated, with the cumulative value being positively correlated with their contribution. To avoid autocorrelation among yield, quality indicators, and their driving factors, principal component analysis (PCA) was first performed in R (v4.1.3) to reduce dimensionality by standardizing multiple similar variables into composite variables. The first principal component (PC1) was then used as a new variable input for structural equation modeling (SEM). Based on relevant literature (Li et al. 2025), model fit indices were screened and the SEM was constructed accordingly.

2.7 Statistical Analysis

Statistical analysis was performed using Excel 2021. One-way analysis of variance (One-way ANOVA) followed by Duncan's multiple range test was conducted for data analysis. Comparisons between groups were performed using Student's t-test for significance analysis. The results were considered significant at the $P < 0.05$ and $P < 0.01$ levels, and highly significant at $P < 0.001$. All data in this study are presented as mean values with standard errors. Half-violin boxplots were generated using the Boxplot package in R (v4.1.3). The SEM diagram was created using PowerPoint 2021, and all other figures were refined and integrated using Adobe Illustrator (AI) 2020.

3. Results

3.1 Analysis of Physico-chemical Soil Properties and Enzyme Activity

The physico-chemical properties and enzyme activities of rhizosphere soil changed significantly during tomato growth and development (Figure 1). Specifically, compared with the CK treatment, SynCom generally increased most soil nutrient indicators (such as EC, AN, S-UE, S-SC, S-CAT and S-ALP). However, significant differences were only observed for AP, S-UE and S-CAT at the seedling and ripening stages. In contrast, soil pH and OM were significantly reduced ($P < 0.05$). These results indicate that SynCom exerts stage-specific effects on the regulation of rhizosphere soil fertility, with the most pronounced impacts on enhancing OM utilization, AP content and S-UE activity.

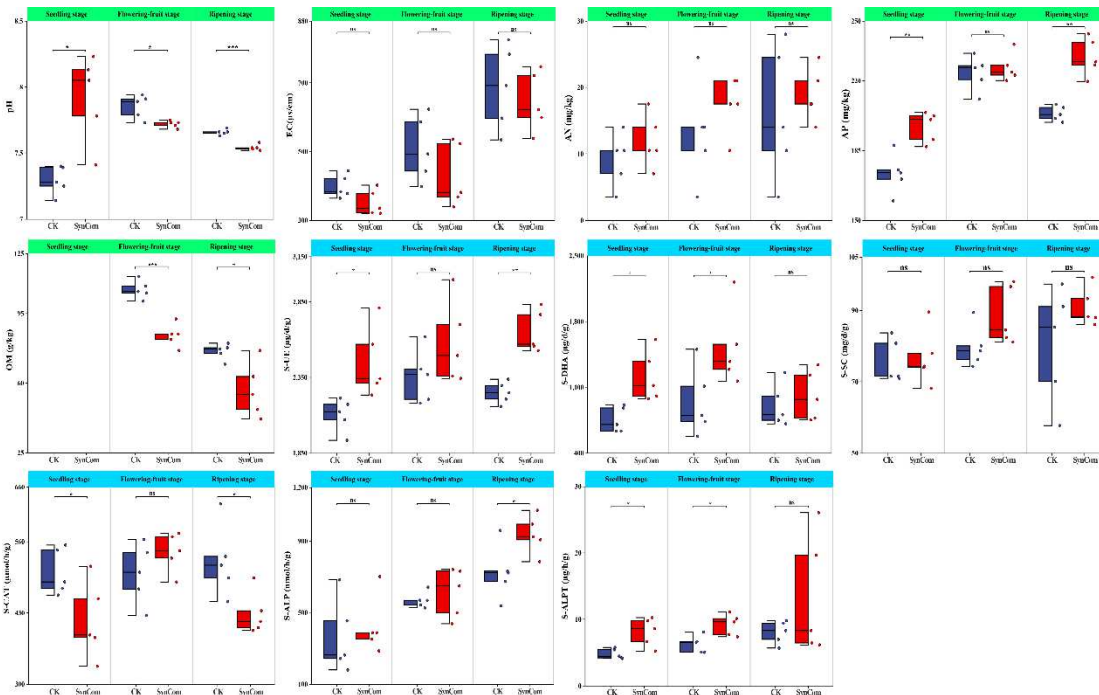


Figure 1. Effects of SynCom on the physico-chemical properties and enzyme activity of tomato rhizosphere soil at different growth stages. *, **, *** and ns represent $P < 0.05$, $P < 0.01$, $P < 0.001$ and not significant, respectively.

3.2 Effects of SynCom on the Diversity of Indigenous Rhizosphere Microbial

Communities

To evaluate the effectiveness of SynCom inoculation in alleviating continuous cropping obstacles, we systematically analyzed the structure of rhizosphere microbial communities in tomato. After Illumina sequencing and quality control, a total of 33,024 bacterial ASVs, 3,733 fungal ASVs, and 5,627 protistan ASVs were obtained from soil samples, indicating high diversity and complexity in the rhizosphere microbiome.

Alpha diversity analysis (Table 1) revealed that bacterial diversity, richness, and evenness generally decreased as the growth stages progressed. Notably, at the ripening stage, the diversity and evenness under SynCom treatment were significantly lower than those of the control, while richness was significantly higher only at the seedling stage. In contrast, fungal communities were most responsive to SynCom at the seedling stage, with diversity, richness, and evenness all significantly higher than in the control. Meanwhile, no significant differences were observed in protistan communities between treatments ($P < 0.05$).

Table 1. The diversity index of microbial communities

	SampleID	Shannon	Chao 1	Pielou_e
Bacteria	CKS	7.29±0.11 ^a	3088.65±169.88 ^b	0.91±0.01 ^a
	SynComS	7.41±0.08 ^a	3348.87±201.15 ^a	0.92±0.01 ^a
	CKF	6.38±0.40 ^a	1875.05±154.73 ^a	0.83±0.03 ^a
	SynComF	6.08±0.25 ^a	2055.66±115.78 ^a	0.80±0.02 ^a
	CKR	5.91±0.39 ^a	1755.17±296.75 ^a	0.79±0.04 ^a
	SynComR	5.49±0.43 ^b	1832.98±301.86 ^a	0.73±0.04 ^b
Fungi	CKS	3.24±0.10 ^b	320.48±44.02 ^b	0.56±0.01 ^b
	SynComS	4.07±0.46 ^a	411.42±39.35 ^a	0.66±0.05 ^a
	CKF	3.88±0.20 ^a	367.71±8.19 ^a	0.66±0.03 ^a
	SynComF	3.97±0.29 ^a	379.75±5.61 ^a	0.67±0.04 ^a
	CKR	3.74±0.28 ^a	339.15±33.41 ^a	0.64±0.04 ^a
	SynComR	3.43±0.22 ^a	325.75±30.93 ^a	0.59±0.03 ^a
Protists	CKS	3.24±0.10 ^a	223.27±3.90 ^a	0.58±0.03 ^a
	SynComS	3.34±0.25 ^a	234.41±11.23 ^a	0.62±0.04 ^a
	CKF	4.20±0.16 ^a	498.13±67.86 ^a	0.68±0.04 ^a
	SynComF	4.33±0.25 ^a	462.75±28.97 ^a	0.68±0.07 ^a
	CKR	5.30±0.13 ^a	594.75±61.42 ^a	0.83±0.01 ^a
	SynComR	5.18±0.21 ^a	462.41±49.53 ^a	0.83±0.01 ^a

Note: The data is presented as mean ± standard deviation, different letters indicate significant differences ($P < 0.05$).

S, F and R represent the seedling, flowering-fruit and ripening stage, respectively.

Beta diversity analysis further revealed the driving mechanisms of microbial community variation. For bacterial communities, PC1 and PC2 explained 34.68% and 8.45% of the variation, respectively (Figure 2A); for fungal communities, 49.55% and

9.32% (Figure 2B); and for protistan communities, 39.57% and 15.97% (Figure 2C). These results indicate that bacterial and fungal community structures were primarily driven by plant growth stages, whereas protistan communities were mainly influenced by treatment effects.

Overall, SynCom exerts stage- and taxon-specific regulatory effects on the rhizosphere microbiome, reflecting distinct response mechanisms of different microbial groups to continuous cropping stress and its alleviation.

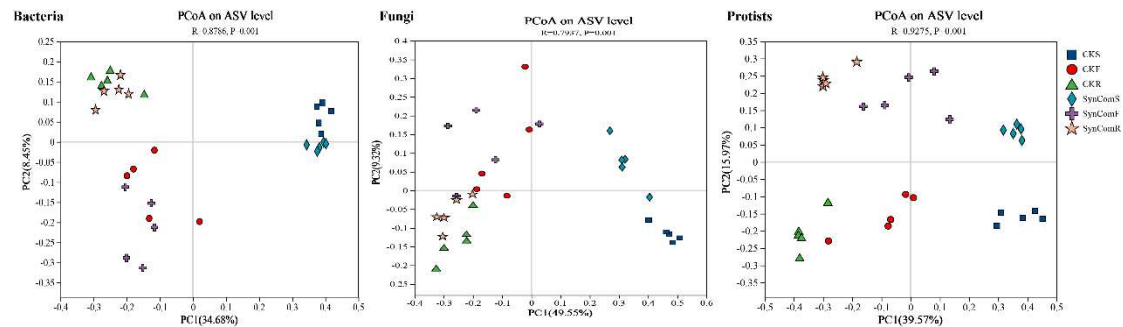


Figure 2. Bray–Curtis similarity analysis of microbial communities. S, F and R represent the seedling, flowering-fruit and ripening stage, respectively.

3.3 Effects of SynCom on the Composition of Indigenous Rhizosphere Microbial

Communities

Across different growth stages, the relative abundances of microbial communities at the phylum level exhibited dynamic changes, while the overall community composition remained similar between treatments (Figure 3). As tomato plants developed, the relative abundances of Proteobacteria and Mortierellomycota increased continuously, with significantly greater increments under SynCom treatment than in the CK ($P < 0.05$). The relative abundances of Firmicutes and Actinobacteriota showed an inverted “U”-shaped pattern (increase then decrease), with no significant differences between treatments. In the fungal community, Ascomycota gradually decreased under SynCom treatment but displayed a “U”-shaped trend in the control. In the protistan community, Cercozoa, Ciliophora, Bigyra, and Apicomplexa all exhibited “U”-shaped trends, with only Ciliophora showing a significant difference between treatments ($P < 0.05$).

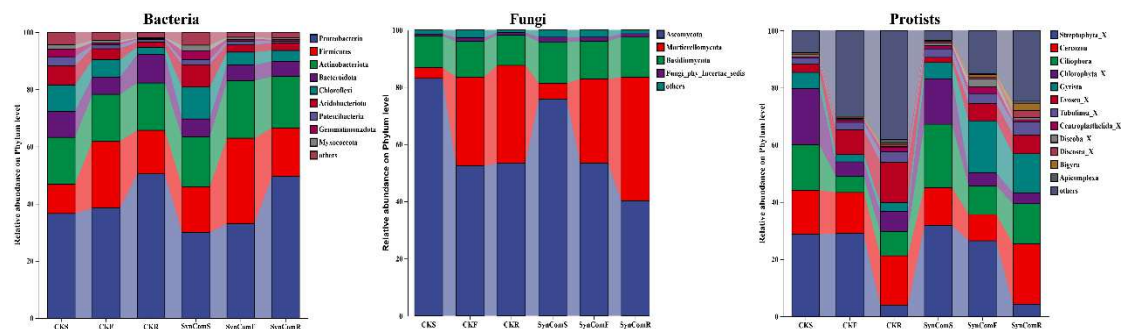


Figure 3. Phylum-level composition of rhizosphere microbial communities.

According to the analysis conducted at ASV level, a differential bar diagram was utilized to illustrate the variations in microbial populations at the genus level across different growth stages of tomato plants, as shown in Figure 4. In the bacterial community, SynCom significantly enriched taxa such as *Bacillus*, *Massilia*, *Azospirillum* and *Streptomyces*. In the fungal community, beneficial taxa including *Trichoderma* and *Mortierella*, as well as the potential pathogen *Botryotrichum*, were significantly enriched. In the protistan community, the predatory genus *Colpoda* was significantly enriched under SynCom treatment ($P < 0.05$).

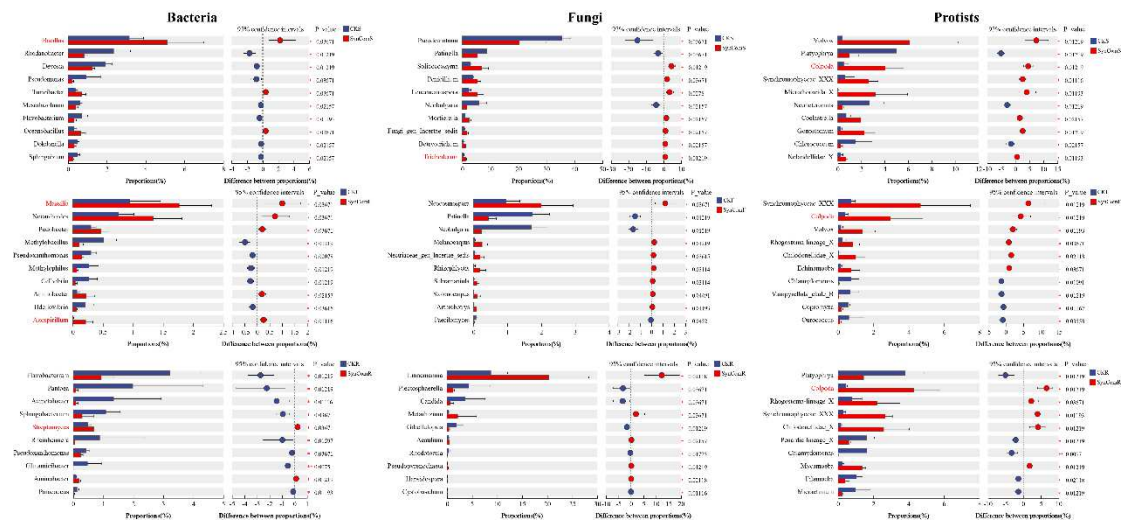


Figure 4. Genus-level composition of rhizosphere microbial communities.

Subsequently, we further investigated the colonization of SynCom strains in the tomato rhizosphere (Figure 5). FN0603, JN25 and aHhLer0J1 each matched a single ASV, whereas fHhLee0B1 and BP16 matched eight and sixteen ASVs, respectively, with distinct temporal dynamics. Over tomato growth, fHhLee0B1 abundance showed an inverted “U”-shaped trend, with significant differences between treatments at the seedling and flowering-fruit stages ($P < 0.05$). BP16 abundance gradually increased, but without significant treatment differences. FN0603 and aHhLer0J1 were exclusively detected in the SynCom treatment, while JN25 abundance steadily declined, differing significantly from the CK control ($P < 0.01$).

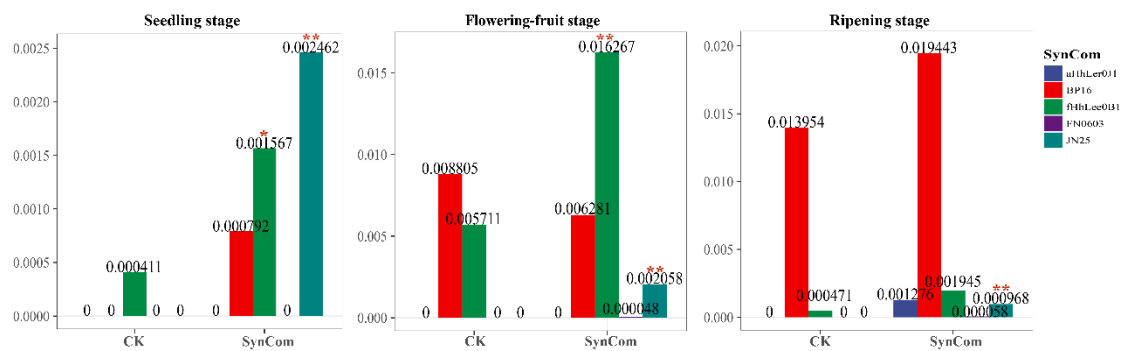


Figure 5. Relative abundance of rhizosphere core microorganism ASV in different treatments. * and ** represent significant differences at $P < 0.05$ and $P < 0.01$, respectively.

3.4 Comprehensive Analysis of Soil Microenvironment, Fruit Traits and Microbial Communities

In this study, Mantel tests and Pearson correlation analyses were performed to evaluate the coupling relationships among soil microbial communities, the soil microenvironment (including enzyme activities and physicochemical properties) and fruit traits (yield, quality attributes and key enzymes involved in sugar metabolism) (Figure 6).

Pearson correlation analysis showed that tomato fruit yield, quality and sugar metabolism-related enzymes were all significantly and positively correlated with one another ($r > 0.57$, $P < 0.001$), whereas among soil physicochemical properties and enzyme activities, only AP and OM exhibited an extremely significant positive correlation ($r = 0.72$, $P < 0.001$), with the remaining variables showing widespread significant correlations. Specifically, yield was strongly and positively correlated with all other fruit traits ($r > 0.88$). Except for titratable acidity (TA; $r = 0.57$), lycopene (Ly) was highly correlated with all other fruit indicators, with correlation coefficients exceeding 0.90. In addition, AP was significantly correlated with urease (S-UE), sucrase (S-SC), and alkaline phosphatase (S-ALP) ($P < 0.01$). Among soil enzymes, catalase (S-CAT) was the only enzyme showing negative correlations with the other enzymes, although none of these relationships reached statistical significance.

Mantel-test results further demonstrated that all microbial groups were significantly associated with the above phenotypic variables, with protistan community showing the strongest correlations with most indicators, followed by fungal community, while bacterial community exhibited comparatively weaker associations. Significant correlations ($P < 0.05$) were consistently observed between all microbial groups and TA, AP and OM. Overall, the strength and extent of the associations were highest for protistan community, intermediate for fungal community, and lowest for bacterial community.

These results indicate that rhizosphere microbial communities, particularly protistan community, are closely coupled with tomato fruit sugar metabolism, quality formation, and yield improvement. The findings suggest that SynCom may promote tomato growth indirectly by improving the soil microecological environment and restructuring microbial community composition. More broadly, these results highlight the critical role of multitrophic interactions in stabilizing crop yield and enhancing fruit quality.

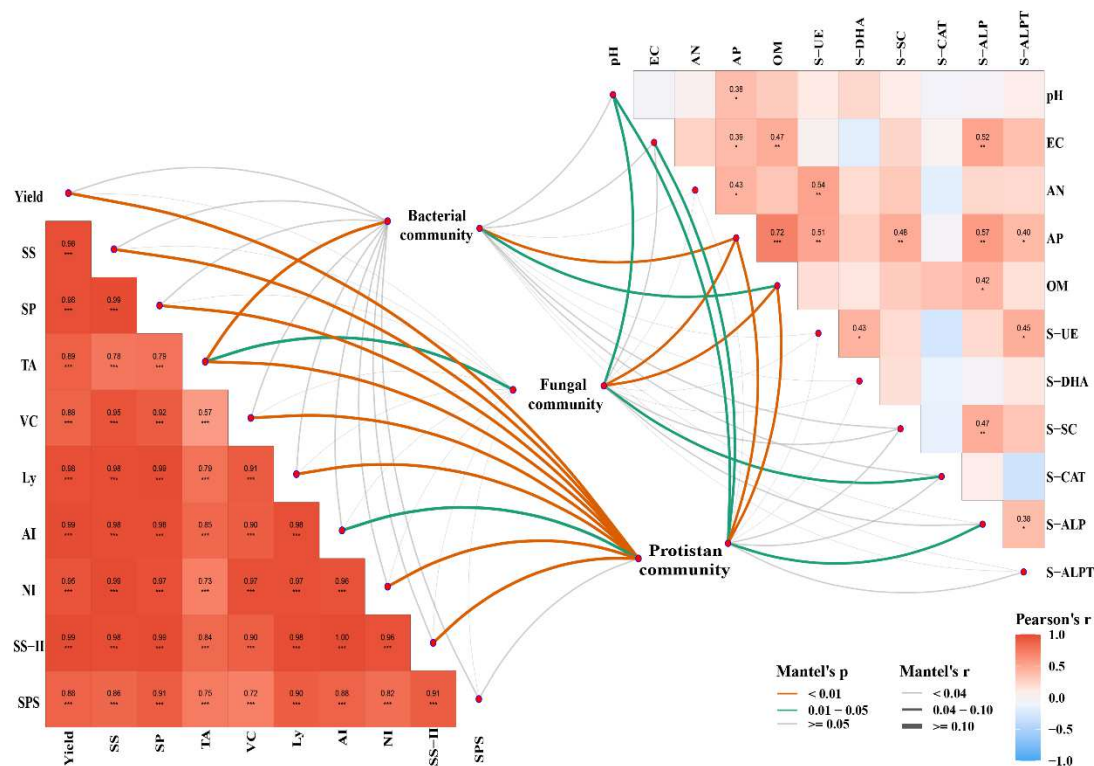


Figure 6. Correlations between soil microenvironment, fruit traits and microbial communities. *, ** and *** represent $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

3.5 Variations in Microbial Communities Contributions to Yield and Quality

Random forest analysis (Table 2) revealed significant differences in the contributions of microbial communities to tomato yield and quality. The protistan community had the highest %IncMse values (84.10 for yield, 125.26 for quality), followed by fungal community (71.02) and bacterial community (100.08), demonstrating that protistan community contributed most to yield (40.48%) and quality (40.92%) and were key predictive groups. Fungi ranked second for yield, while bacterial community surpassed fungal community for quality. Overall, protistan community may indirectly regulate yield and quality via interactions with other microbes in soilborne disease suppression, nutrient cycling, and root health maintenance. These findings highlight the need to emphasize the role of it in alleviating continuous cropping obstacles and their underlying regulatory mechanisms.

Table 2. The diversity index of microbial communities

	Bac	Fun	Pro	Bac	Fun	Pro
	/ %IncMse	/ %IncMse	/ %IncMse	contribution	contribution	contribution
Yield	52.64	71.02	84.10	25.34%	34.18%	40.48%
Quality	100.08	80.77	125.26	32.69%	26.39%	40.92%

Note: Bac, Fun and Pro represent the bacterial community, fungal community and protistan community, respectively.

3.6 Microbial Network Analysis

Co-occurrence network analysis was performed to explore the interaction between different microbial taxonomies under different treatments. Compared with CK, the SynCom treatments of bacterial and protistan communities exhibited higher edge counts, edge density, average degree, and lower average path length, whereas the fungal community showed the opposite trend. The bacterial community also demonstrated higher average clustering coefficient and relative modularity. These results indicate that inoculation with the synthetic community enhanced the frequency of potential interactions among bacterial taxa, resulting in a more compact network structure and a clearer functional compartmentalization (Table 3, Figure S2).

Table 3. Topological characteristics of cross-domain networks of microbial communities

Network type	Edge number	Connectance (Edge density)	Average degree	Average path length	Average clustering coefficient	Relative modularity
Bac-CK	1567	0.20	24.68	1.77	0.61	0.24
Bac-SynCom	1798	0.22	28.09	1.56	0.63	0.83
Fun-CK	718	0.16	15.28	1.96	0.67	0.89
Fun-SynCom	532	0.15	12.52	2.15	0.62	0.42
Pro-CK	1200	0.21	22.22	1.79	0.69	0.87
Pro-SynCom	1733	0.28	30.95	1.72	0.68	0.20
Bac-Fun	817	0.12	14.21	2.44	-	-
Fun-Pro	674	0.11	12.14	2.45	-	-
Bac-Pro	1336	0.17	21.04	2.32	-	-

Note: Bac, Fun and Pro represent the bacterial community, fungal community and protistan community, respectively.

- represents no test data.

To further investigate the potential associations among different microbial kingdoms, we constructed cross-domain correlation networks (Figure 7). The results showed that the interactions between protistan and bacterial communities were the highest (e.g., exhibiting more edges and a lower average path length), followed by those between bacterial and fungal communities, while the interactions between fungal and protistan communities were the weakest (Table 3). These findings suggest that bacterial and protistan communities may form closer linkages. The topological characteristics of the cross-domain networks (Table 3) also indicated that the bacterial-protist network exhibited the highest edge count, edge density, and average degree, suggesting a higher degree of association and a more complex and tightly interconnected interaction network between these two groups. This suggests that bacterial-protist interactions may play a key role in the formation of continuous cropping obstacles and their associated ecological consequences.

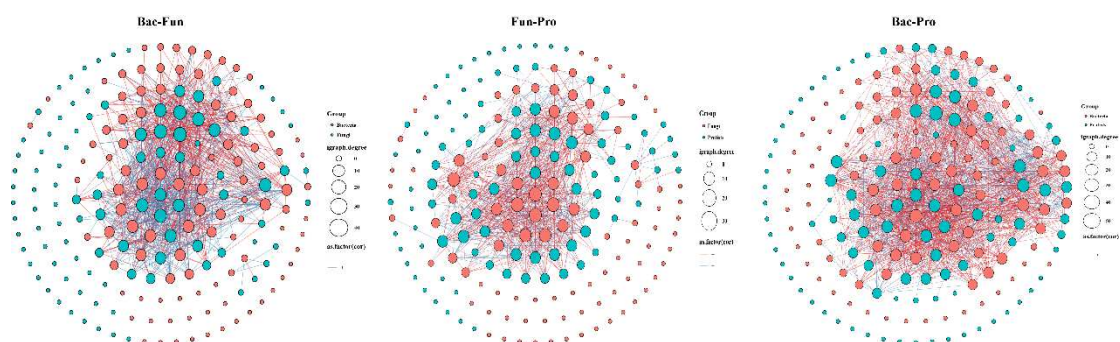


Figure 7. Cross-domain network diagram of multi-trophic microbial communities. Bac-Fun, Fun-Pro and Bac-Pro respectively represent the interaction between bacterial-fungal communities, fungal-protistan communities, and bacterial-protistan communities, respectively.

3.7 Structural Equation Modeling (SEM) Analysis

To further distinguish the direct and indirect effects of multiple factors on fruit yield and quality, we conducted a SEM analysis and hypothesized the relationships between them (Figure 8). The results showed that the probability level was 0.541 (> 0.05), indicating a good fit of the SEM model. Most of the pathways were positively correlated. Specifically, SynCom had direct and significant positive effects on plant growth, rhizosphere soil physicochemical properties and yield ($P < 0.01$), with regression weights of 0.560, 0.606 and 0.110, respectively. In contrast, SynCom exhibited a direct but negative effect on the fungal community, although this effect did not reach a statistically significant level (Figure 8a). Furthermore, SynCom showed the highest standardized total effects on crop yield and quality, with values of 0.699 and 0.760, respectively (Figure 8b&c). Overall, the positive effect of SynCom on fruit quality was not direct, but was mediated indirectly through its influences on plant growth, soil properties, and microbial communities. By comparison, the effect of SynCom on yield was more direct, although the indirect effect remained greater than the direct effect.

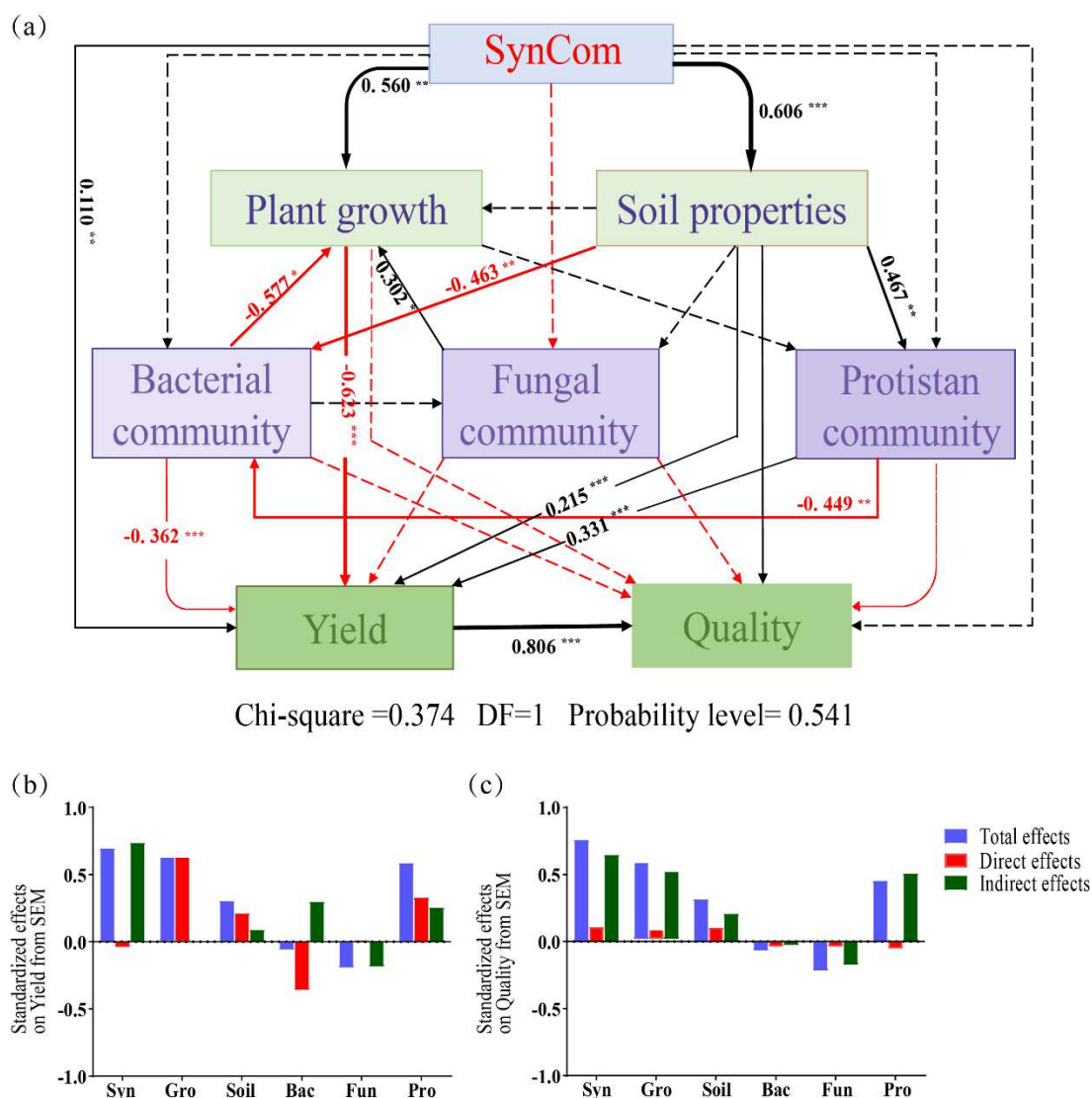


Figure 8. Structural equation modeling (SEM) demonstrates the overall impact of multiple indicators on yield and quality standards. The black and red solid lines and their thicknesses respectively represent positive and negative correlations and regression weights. The dotted lines indicate no significant correlation. In (b) and (c), Syn, Gro, Soil, Bac, Fun and Pro represents SynCom, plant growth, soil properties, bacterial community, fungal community and protistan community, respectively.

4. Discussion

Continuous cropping obstacles (CCOs) represent a pervasive soil degradation issue in facility agriculture, and their development is closely linked to imbalanced soil nutrients, accumulation of autotoxic substances, proliferation of soilborne pathogens, and disruption of the rhizosphere microecology (Xu et al. 2025). The resulting microecological dysbiosis, which ultimately leads to declines in crop yield and quality, is widely regarded as a hallmark manifestation of CCOs (Zhang et al. 2025; Yu et al. 2024), with deterioration of the soil microbial habitat being the underlying culprit (Chen et al. 2022). Consequently, ameliorating soil microecology is the primary and most sustainable effective strategy for alleviating or eliminating CCOs in crops (Zhang et al. 2020). Such improvement not only promotes plant growth but also reinvigorates the

indigenous microbial communities. Although the application of microbial inoculants has achieved substantial progress in mitigating continuous cropping issues, a clear understanding of how they distinctly modulate soil nutrient cycling, reshape microbial community structure, and ultimately influence crop yield and fruit quality remains elusive, particularly in greenhouse tomato production.

This study found that the most direct response after SynCom inoculation was manifested as improvements in soil nutrient cycling and enzyme activities. The significant decreases in pH and soil organic matter, coupled with a marked increase in available phosphorus (Figure 1), may be attributed to the active secretion of organic acids by functional strains within the SynCom (e.g., *Pseudomonas* spp.) to acquire nutrients, thereby accelerating the decomposition and mineralization of organic matter and facilitating the release of immobilized phosphorus into plant-available forms (Hao et al. 2024). These findings are consistent with those of Wei et al. (2024), who demonstrated that microbial inoculants play a critical role in alleviating soil acidification and nutrient imbalance. Regarding soil enzyme activities, the significant increases in urease and protease activities indicated an accelerated nitrogen cycle, while the elevated dehydrogenase activity directly confirmed enhanced overall metabolic activity of the soil microbial community. In contrast, the marked decrease in catalase activity reflected a progressive stabilization of the soil microecology (Wen et al. 2025; Silva et al. 2023) ($P < 0.05$). Notably, the significant decrease in soil organic matter, concurrent with increased available nutrients and improved plant growth, suggests that the decline in organic matter resulted from accelerated microbial decomposition and utilization rather than from soil degradation. Similar phenomena were observed by Wang et al. (2025) in a study on continuous cropping tomato soil improvement, where the application of a combined amendment containing *Bacillus subtilis* and *Trichoderma harzianum* significantly enhanced soil available phosphorus and phosphatase activity.

In addition to soil environmental factors, SynCom also significantly influenced the rhizosphere microbial community structure. Diversity is a key indicator of soil microbial community stability and functional potential. Beta diversity analysis revealed that bacterial and fungal communities were primarily driven by growth stage, whereas protist communities were more sensitive to SynCom inoculation, indicating differential response mechanisms among microbial communities across trophic levels (Figure 2). Previous studies have found consistent results that the developmental stage of plants is an important factor driving the differentiation of bacterial and fungal communities (Schlemper et al. 2018; Li et al. 2022b), and exogenous microbial inoculation can significantly alter the structure of native microbial communities and further affect high trophic level microorganisms by changing the bacterial/eukaryotic ratio (Xiong et al. 2019). In this study, the significant increases in fungal diversity and bacterial richness at the seedling stage (Table 1) suggested that SynCom not only enhanced bacterial richness but also promoted early colonization of beneficial fungi and functional complementarity within the community. Although bacterial diversity decreased during the mature stage, yield and quality improved significantly, suggesting that functional optimization outweighs sheer diversity under continuous cropping stress (Banerjee et al. 2023). This trend suggests that SynCom stimulated the diversity of indigenous

microorganisms in the early stages through resource competition or niche grabbing; The intensification of competition in the later stage leads to community homogenization (Carlström et al. 2019).

At the taxonomic composition level, SynCom significantly enriched multiple microbial taxa possessing both plant-growth-promoting and biocontrol traits (Figure 4), including *Bacillus* and *Streptomyces* that produce antibacterial substances (Rabbee et al. 2021), *Azospirillum* that secretes auxin (Tagoe et al. 2026), *Trichoderma* that induces plant systemic resistance, and *Mortierella* that promotes phosphorus activation (Yao et al. 2023; Sang et al. 2022). The co-enrichment of these functional groups suggests that SynCom not only exerts direct beneficial effects but also recruits indigenous beneficial microorganisms to participate in rhizosphere ecological regulation, thereby establishing a more stable and efficient microbial interaction network. The prerequisite for exogenous strains to function is whether they can stably colonize (Marín et al. 2021). The dynamic colonization results of inoculated strains further demonstrate that SynCom has good adaptability (Figure 5). For instance, strains aHhLer0J1 and FN0603 were detected exclusively in the SynCom treatment, while JN25 maintained consistently high abundance throughout the entire growth period. There was a temporal functional division of labor among SynCom strains (early root morphogenesis, later enhanced nutrient supply and disease suppression), which helps maintain the long-term stability and ecological function of SynCom. The potential pathogenic fungus *Botryotrichum* was also enriched in SynCom, suggesting a dual regulatory role of the inoculant.

The marked enrichment of the predatory protist Colpoda is a key finding, further corroborating the reshaping of the tomato rhizosphere microbiome by SynCom. Previous studies have demonstrated that protist predation pressure can promote nutrient release, stimulate the turnover and functional expression of plant-growth-promoting bacteria (shifting interactions from competition to cooperation), and indirectly enrich beneficial microorganisms, thereby enhancing plant health and suppressing soilborne diseases (Gao et al. 2019; Liu et al. 2026). Although protists have long been neglected in agricultural microbiological research, a growing body of evidence now recognizes them as crucial regulators of soil ecological functions (Geisen et al. 2018; Guo et al. 2018). Both Mantel tests and random forest model analyses further indicated that the protist community exhibited the strongest and most significant correlations with and predictive power for tomato yield and quality. Collectively, the primary mechanism by which SynCom improves crop performance is not through its direct growth-promoting effects, but rather indirectly, by modulating the indigenous protist community and consequently enriching specific bacterial taxa (e.g., *Bacillus* spp.).

This result was further corroborated by microbial co-occurrence network analyses. SynCom inoculation led to more frequent potential inter-bacterial associations, a more compact network architecture, and more distinct functional modules (Table 3, Figure S2), while the cross-kingdom networks between bacteria and protists became more complex and tighter. This indicates that the introduced SynCom enhances the disturbance resistance and stability of tomato rhizosphere ecosystems by promoting interactions between rhizosphere microbial communities, improving nutrient cycling,

and inhibiting pathogens (Yadav et al. 2025). The interaction of multi trophic level microorganisms is a key mechanism for maintaining rhizosphere homeostasis and enhancing the stress resistance of agricultural ecosystems. Rossmann et al. (2020) conducted a systematic analysis of the wheat rhizosphere microbial community and found that high trophic level organisms have a regulatory effect on microbial community assembly, and that positive associations between protists and bacteria play an important role in maintaining rhizosphere microecological stability. Li et al. (2026) further elucidated that soil fauna shape functionally complementary beneficial bacterial assemblages via predation pressure, enhancing the stability of the microbiome against pathogen invasion, and proposed that multi-trophic positive feedback loops constitute the core mechanism underpinning rhizosphere disease-suppressive functionality. Additionally, the study by Shi et al. (2024) in tomato systems also confirmed that rhizosphere microbial diversity and network complexity have a core contribution to improving plant nutrient absorption and stress response capabilities. Structural equation modeling (SEM) analysis further elucidated the multi-pathway regulatory mechanisms through which SynCom promoted tomato growth, yield, and quality improvement. SynCom had a predominantly direct effect on yield (standardized total effect = 0.699), while also exerting significant positive direct effects on plant growth and soil properties (regression weights = 0.560 and 0.606, respectively). In contrast, the promotion of fruit quality by SynCom was mainly indirect, achieved through the modulation of plant growth, soil properties, and microbial communities (standardized total effect = 0.760). This suggests that the increase in yield primarily resulted from enhanced root growth and nutrient uptake efficiency, whereas quality formation was closely associated with sugar metabolism and carotenoid accumulation. The highly significant positive correlations between key enzymes involved in sugar metabolism and both yield and quality indicators (Figure 6) further supported this notion. In summary, the simplified indigenous SynCom effectively alleviates tomato CCOs through a cascading pathway: it improves rhizosphere properties, modulates higher-trophic-level native communities, recruits beneficial microorganisms, enhances community stability, and ultimately boosts yield and quality. Wang et al. (2025) observed the same complete pathway in the study of continuous cropping tomatoes using amendments. Other studies have revealed the intrinsic relationship between microbial community stability and crop yield from a multi trophic perspective, indicating that diversity of key microbial communities at multiple trophic levels is the core of maintaining soil functional potential (Fan et al. 2020 & 2021). Xiao et al. (2024) further demonstrated from a network analysis perspective that complex microbial interaction networks have stronger resistance to pathogen invasion and are positively correlated with ecosystem stability. In addition, the findings of Crawford et al. (2020) in ecological restoration systems also provide evidence. The above research collectively supports the multi-layered cascade regulatory pathway revealed in this study from different perspectives.

5. Conclusion

This study demonstrated that an indigenous synthetic microbial community (SynCom) can effectively alleviate continuous cropping obstacles in greenhouse tomato production through stage-specific regulation of the rhizosphere microecological environment. SynCom inoculation significantly increased soil available phosphorus content as well as urease and catalase activities, reshaped the rhizosphere microbial community structure, and enriched beneficial microorganisms, including *Bacillus*, *Azospirillum*, *Streptomyces* and *Trichoderma*. Integrated correlation, random forest, network, and SEM analyses consistently identified protistan communities and their bacterial interactions as key ecological drivers of tomato yield and fruit quality improvement. In particular, the positive effects of SynCom on fruit quality were primarily mediated indirectly through the regulation of plant growth, soil properties, and multitrophic microbial interactions. Overall, the indigenous SynCom alleviated continuous cropping stress by optimizing the rhizosphere microecological network, providing a theoretical basis for developing region-specific microbial inoculants and promoting sustainable protected agriculture.

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CRedit authorship contribution statement

Yu Zhou: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing. Yuze Guo: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. Yang Liu: Conceptualization, Formal analysis, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing. Yadi Song: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Supervision, Validation, Visualization, Writing – review & editing. Ting Yao: Conceptualization, Data curation, Formal analysis, Investigation, Software, Supervision, Validation, Visualization. Lihua Zhang: Conceptualization, Formal analysis, Investigation, Methodology, Supervision, Writing–review & editing. Tang Kai: Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Writing–review & editing. Fuying Feng: Conceptualization, Formal analysis, Funding acquisition, Methodology, Project administration, Resources,

Software, Supervision, Validation, Visualization, Writing—review & editing.

Data availability

Data will be made available on request. The raw sequences have been submitted to the National Center for Biotechnology Information (NCBI, Bethesda, MD, USA) under the accession number PRJNA1442407, PRJNA1442661 and PRJNA1442683, respectively.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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